888-Pos Board B688
Gentle High Speed Imaging of Live Cells with AFM
Paul Ashby.
Deformation of the cell surface hinders the ability of AFM to image at molecular resolution. The damping of the cantilever determines the minimum applied force. We have developed submicron cantilevers and sensitive detection systems to reduce the damping and image at highest resolution. We have also developed active scan algorithms and data augmentation methods which enable high-speed data acquisition for capturing protein dynamics in the membrane.

889-Pos Board B689
Designing Novel Proteins with Enhanced Mechanical Strengths
Wenzhe Lu, Andres F. Oberhauser, Werner Braun.
Designing novel proteins with specific properties remains a difficult goal to achieve, mainly because the understanding of the relationship between protein sequences and certain structures or properties is still incomprehensive. For the case of mechanical proteins, even though few successes are accomplished to enhance the mechanical strengths of protein domains, there are no rational and systematic methods to design proteins with specific mechanical properties. It has been shown that the Ig-like domains in the I-band of Titin need hierarchical external forces to unfold. Dr. Braun's group analyzed the sequences of the weak and strong Ig-like domains from different species, and identified unique motifs which may be important for the hierarchical strengths of Ig-like domains. In this study,we designed four novel proteins by swapping motifs or segments from strong domain I27 to weak domain I1. The hypothesis is that the unfolding forces of the mutants will be increased. We tested their unfolding forces by single molecular Atomic Force Microscope (AFM) experiments to verify the enhancements of mechanical strengths. Preliminary data indicate that the unfolding forces of some designed mutants are increased to the level of I27. Steered Molecular Dynamics (SMD) simulations for these mutants are in process to analyze the conformational changes during unfolding. The change of backbone hydrogen bonds of A-B, A'-G strands and their side-chain interactions with neighboring residues are monitored, since they were reported to be the key determinants of mechanical strengths for Ig-like domains in titin.

890-Pos Board B690
Effects of Electrostatics on Interactions of Short Peptide from Yeast Prion Protein Sup35
Alexander M. Portillo, Alexey V. Krasnoslobodtsev, Yuri L. Lyubchenko.
Protein misfolding and subsequent aggregation are the first steps in a long, complex process central to prion diseases. Previous experiments show distinct growth patterns at different pH values, with the fastest kinetics observed at pH 5.6, which is in line with the AFM force spectroscopy studies revealing the strongest interactions at pH 5.6. Since this pH corresponds to the peptide isoelectric point, we hypothesize that the observed effects are due to electrostatic interactions.
Here, we studied the effect of electrostatics on the aggregation and peptide-peptide interactions of a short fragment of the yeast prion protein, Sup35 (GNNQQNY), used as a model to describe protein misfolding. We characterized aggregate growth using AFM and Thioflavin T (ThT) fluorescence measurements in various pHs and ionic strengths. We used single molecule AFM force spectroscopy to characterize peptide-peptide interactions at different ionic strengths. The electrostatic effect should be the strongest at pH values far from the pI and the lowest at the isoelectric point. Indeed, the experiments showed that at pH 2.0, the aggregation lag time is 195 hours under low ionic strength (10 mM) whereas the lag time decreases to 42 hours when ionic strength at pH 2.0 increases to 150 mM. At pH 5.6, the aggregation lag time is 11 hours under low ionic strength, and there is minimal change to lag time when ionic strength is increased. In parallel, AFM force spectroscopy studies were performed. They revealed minimal contribution of electrostatics in the dissociation of transient dimers. Increasing ionic strength at pH 2.0 resulted in a significant increase in forces. Such an increase in the magnitudes of rupture forces indicates that ionic strength enhanced inter-peptide interactions at low pH. The effect was less noticeable near the isoelectric point.

891-Pos Board B691
Single Molecule Study of Cis-Platin DNA Interaction
Samrat Dutta.
Background: Cis-platin belongs to the family of inorganic metal drugs which has a success rate of ~ 90 % against testicular and ovarian cancer. Substantial research has been done on cisplatin-DNA interaction using various techniques including atomic force microscopy AFM, however, the exact mechanical change (e.g. bend angle and ridigidty) in DNA due to its interaction with a single molecule of cis-platin is controversial. Elucidation of these parameters will enhance our understanding of how proteins interact with cis-platin and will, thus, enhance our understanding of the successes and limitations of cis-platin.
Objectives & Methods: Using statistical analysis of AFM imaging and simulations based on the worm-like-chain model we investigated the interaction of cis-platin with DNA at the single molecule level to determined the cis-platin induced DNA bend angle and change in DNA mechanical properties. We used three constructs of DNA (300 bp) with a single GG, a single AG or two, in-phase GG sites in the center. We measured the contour length (L), end to end distance(R) of DNA and DNA-cisplatin complexes, and determined the persistence length (P), bend angle β, and local flexibility around the cis-platin site.
Results: R/L Distribution of the DNA-cisplatin complex showed a small but significant change when compared with the normal DNA distribution; the cis-platin-induced bend angle β was measured to be ~38°. R/L distribution of the simulated DNA molecules matched the experimental data.
Conclusions: The R/L distribution of the DNA-cisplatin complex vs normal, of AFM images and simulated DNA molecules shows that the DNA is damaged locally at the platination site while retaining its global mechanical properties. Our bend angle agrees with the X-ray and the gel-electrophoresis data further confirming the specificity of our technique in capturing the DNA-cisplatin interaction.

892-Pos Board B692
An Atomic Force Microscopy Study of Live Cells and Biofilms of Rhizobium Leguminosarum
Jun Dong, Elizabeth M. Vanderlinde, Christopher K. Yost, Tanya E.S. Dahms.
The bacterial cell envelope acts as stress-bearing component to provide the first layer of protection for bacteria from exposure to antibiotics, dyes, and detergents in their environment. Biofilms, aggregates of bacteria embedded in the extracellular polysaccharide, provide further protenction for bacterial survival. Atomic force microscopy has the ability to probe the surface structure of bacteria at high resolution, but it is still a significant challenge to develop sample preparation techniques for live cell imaging. In the present study, we adapt a sample preparation method for imaging live bacteria by atomic force microscopy, which allows the bacteria exposed to an ambient environment. Soil bacteria Rhizobium leguminosarum wide type 3841 and its carboxy terminal protease (ctpA) mutant R. leguminosarum 3845 (Gilbert et al. (2007) FEMS Microbiol Lett. 272(1):65-74) were examined using this technique. The wide type and mutant rhizobia show significant differences in morphology, surface ultrastructure, and biofilm formation. Force spectroscopy was used to probe the elasticity and relative surface chemistry of the mutant and wild type rhizobia, both as individual cells and as part of a biofilm, each showing distinct differences.

893-Pos Board B693
Astrocyte Cell-Cell Interactions via Long-Range Connective Bridges on Directive Surfaces
Volkan M. Tiryaki, Virginia M. Ayres, Adeel A. Khan, Dexter A. Flowers, Sally Meiners, Ijaz Ahmed, Roberto Delgado-Rivera.
In previously reported work [1], we demonstrated that astrocytes cultured on synthetic polyamide nanofibrillar surfaces that mimic the architecture of the capillary basement membrane assumed morphological forms that recapitulated their physiology within the developing central nervous system. In the present work, atomic force microscopy was used to investigate astrocyte cell-cell interactions at 24 h, for cells cultured on nanofibrillar versus planar surfaces. For the nanofibrillar surfaces, high pass spatial filtering was required to distinguish the nanofibrillar background from the nanoscale astrocyte features. Using this approach, details of the physical interactions between astrocytes on nanofibrillar surfaces via connective extensions across ~50 μm distances were identified, which were not observable in epi-fluorescent microscopy, or in tapping or deflection mode atomic force microscopy. Astrocyte cell-cell interactions were shown to differ in connective extension type, cell body type, and number of interactions. The connective bridges took the form of a filopodia network for planar surfaces but a single extension lamellipodia bridge for the nanofibrillar surfaces. Structures suggestive of adherens versus gap junctions that were part of the connective extensions were also identified. Cell-cell interactions via connective bridges (filopodia bridges, or tunneling nanotubes) over distances much larger than adjacent cell wall-cell wall contact distances have been previously reported for planar substrates. The present research supports this work and adds the dimension that nanofibrillar versus planar surface architectures can also be directive for specific implementations of such long-distance interactions.
[1] Delgado-Rivera, R, Harris, SL, Ahmed, I, Babu, AN, Patel, R, Kamal, J, Ayres, V, Flowers, D, Meiners, S, 2009. Increased FGF-2 secretion and ability to support neurite outgrowth by astrocytes cultured on polyamide nanofibrillar matrices. Matrix Bio. 28: 137-147.